STN 3/1/96

08/333,680

(FILE 'HOME' ENTERED AT 07:48:45 ON 01 MAR 96)

FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE' ENTERED AT 07:49:11 ON 01 MAR 96

L1 1363 FILE CAPLUS
L2 1219 FILE EMBASE
L3 1323 FILE BIOSIS
L4 1477 FILE MEDLINE
TOTAL FOR ALL FILES
L5 5382 S (ADENO?) AND VECTOR#
L6 10999 FILE CAPLUS
L7 5851 FILE EMBASE
L8 5689 FILE BIOSIS
L9 5832 FILE MEDLINE
TOTAL FOR ALL FILES
L10 28371 S SECOND AND GENERATION
L11 6 FILE CAPLUS
L12 7 FILE EMBASE
L13 6 FILE BIOSIS
L14 7 FILE MEDLINE
TOTAL FOR ALL FILES
L15 26 S L5 AND L10
L16 13 DUPLICATE REMOVE L15 (13 DUPLICATES REMOVED)
L17 2236 FILE CAPLUS
L18 1810 FILE EMBASE
L19 1620 FILE BIOSIS
L20 1710 FILE MEDLINE
TOTAL FOR ALL FILES
L21 7376 S "E4"
L22 28 FILE CAPLUS
L23 17 FILE EMBASE
L24 16 FILE BIOSIS
L25 16 FILE MEDLINE
TOTAL FOR ALL FILES
L26 77 S L5 AND L21
L27 33 DUPLICATE REMOVE L26 (44 DUPLICATES REMOVED)
L28 209 FILE CAPLUS
L29 161 FILE EMBASE
L30 173 FILE BIOSIS
L31 183 FILE MEDLINE
TOTAL FOR ALL FILES
L32 726 S (L21 AND ADENO?) NOT L26
L33 42 FILE CAPLUS
L34 36 FILE EMBASE
L35 31 FILE BIOSIS

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L36          38 FILE MEDLINE
     TOTAL FOR ALL FILES
L37         147 S L32 AND PY>=1992 AND PY<=1994
L38          53 DUPLICATE REMOVE L37 (94 DUPLICATES REMOVED)
L39         512 FILE CAPLUS
L40         488 FILE EMBASE
L41         505 FILE BIOSIS
L42         493 FILE MEDLINE
     TOTAL FOR ALL FILES
L43        1998 S ALPHA AND INHIBIN
L44          15 FILE CAPLUS
L45          12 FILE EMBASE
L46          13 FILE BIOSIS
L47          13 FILE MEDLINE
     TOTAL FOR ALL FILES
L48          53 S L43 AND PROMOTER#
L49          19 DUPLICATE REMOVE L48 (34 DUPLICATES REMOVED)
L50         705 FILE CAPLUS
L51         364 FILE EMBASE
L52         374 FILE BIOSIS
L53         408 FILE MEDLINE
     TOTAL FOR ALL FILES
L54        1851 S CAMP AND RESPONSE AND ELEMENT# AND PROMOTER#
L55          83 FILE CAPLUS
L56          66 FILE EMBASE
L57          53 FILE BIOSIS
L58          88 FILE MEDLINE
     TOTAL FOR ALL FILES
L59         290 S L54 AND ADENO?
L60          71 FILE CAPLUS
L61         117 DUPLICATE REMOVE L59 (173 DUPLICATES REMOVED)
L62          28 FILE CAPLUS
L63          16 FILE EMBASE
L64          18 FILE BIOSIS
L65          19 FILE MEDLINE
     TOTAL FOR ALL FILES
L66          81 S L54 AND ADENOV?
L67          30 DUPLICATE REMOVE L66 (51 DUPLICATES REMOVED)
L68           5 FILE CAPLUS
L69           5 FILE EMBASE
L70           3 FILE BIOSIS
L71           3 FILE MEDLINE
     TOTAL FOR ALL FILES
L72          16 S CAMP AND PROMOTER AND VECTOR AND ADENOV?
L73           6 DUPLICATE REMOVE L72 (10 DUPLICATES REMOVED)
L74           6 FILE CAPLUS
L75          27 FILE CAPLUS
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L16 ANSWER 6 OF 13 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 4
AN 1995:629301 CAPLUS
DN 123:75873
TI Complementation of a human ***adenovirus*** early region 4 deletion mutant in 293 cells using ***adenovirus*** -polylysine-DNA complexes
AU Scaria, A.; Curiel, D. T.; Kay, M. A.
CS Markey Molecular Medicine Center, University Washington, Seattle, WA, 98195, USA
SO Gene Ther. (1995), 2(4), 295-98
CODEN: GETHEC; ISSN: 0969-7128
DT Journal
LA English
AB The E1 deleted ***adenoviral*** ***vectors*** are efficient at gene transfer to cells in culture or in animals. However, their use is limited because of an immune-mediated loss of transduced cells. This immune response is believed to result from low-level prodn. f viral antigens from these ***vectors*** after gene transfer. The early region 4 (E4) of ***adenovirus*** produces a no. of proteins that play an important role in ***adenoviral*** and host gene regulation during infection of mammalian cells. There is interest in developing E4 deficient ***adenovirus*** for gene therapy, esp. in the context of developing a combined E1/E4 deleted ***vector*** . Towards this goal, a method by which to complement and propagate an E4 deficient ***adenovirus*** (dl 1014) in the E1 complementing 293 cell line, using a novel and simple rescue technique, has been developed. Purified ***adenovirus*** deficient in E4 gene expression (dl 1014) was conjugated to expression plasmids contg. the E4-open reading frame 6 gene or complete E4 region to produce ***adenovirus*** -polylysine-DNA complexes that were used to transfect 293 cells. The derived virus obtained from this transfection did not replicate on 293 cells but did replicate on W162 cells (E4+) confirming that the virus was indeed deleted for E4. Viral yield was high ranging from 3 .times. 107 to 9 .times. 108 plaque forming units per 106 293 cells. This method has general application to the prodn. of new ***adenoviral*** mutants that will be useful for developing ***second*** ***generation*** ***adenoviral*** ***vectors*** .

L16 ANSWER 8 OF 13 CAPLUS COPYRIGHT 1996 ACS
AN 1994:595933 CAPLUS
DN 121:195933
TI Gene therapy for cystic fibrosis with ***adenovirus*** -based ***vectors*** encoding the CFTR protein
IN Gregory, Richard J.; Armentano, Donna; Couture, Larry A.; Smith, Alan E.
PA Genzyme Corp., USA
SO PCT Int. Appl., 168 pp.
CODEN: PIXXD2
PI WO 9412649 A2 940609
DS W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US11667 931202
PRAI US 92-985478 921203
US 93-130682 931001
US 93-136742 931013
DT Patent
LA English
AB ***Adenovirus*** -based ***vectors*** are disclosed for use in gene therapy, esp. for cystic fibrosis. Advantages of ***adenovirus*** -based ***vectors*** for gene therapy are (1) they appear to be relatively safe, (2) can be manipulated to encode the desired gene product, (3) at the same time are inactivated in terms of their ability to replicate in a normal lytic viral life cycle, and (4) have a natural tropism for airway epithelia. One such ***adenovirus*** -based ***vector*** comprises an ***adenovirus*** 2 serotype genome in which the E1a and E1b regions were deleted and replaced by genetic material of interest (e.g., DNA encoding the cystic fibrosis transmembrane regulator protein). The ***vectors*** can also encompass pseudo-***adenoviruses*** (PAV), which comprise ***adenovirus*** 2 inverted repeats and the minimal sequences of a wild-type ***adenovirus*** type 2 genome necessary for efficient replication and packaging. PAVs contain no potentially harmful viral genes, have a theor. capacity of foreign material of nearly 36 kb, may be produced in reasonably high titers and maintain the tropism of the parent ***adenovirus*** for dividing and non-dividing human target cell types. Such a ***second*** - ***generation*** ***vectors*** contains the open reading frame 6 (ORF6) of ***adenovirus*** early region 4 (E4) and is deleted for all other E4 open reading frames. Optionally this ***vector*** can include deletions in the E1 and/or E3 regions. Alternatively, the ***adenovirus*** -based gene therapy ***vector*** contains the ORF3 of ***adenovirus*** E4 and is deleted for all other E4 open reading frames; this ***vector*** can also include deletions in

the E1 and/or E3 regions. The deletion of non-essential open reading frames of E4 increases the cloning capacity by .apprx.2 kb without significantly reducing the viability of the virus in cell culture. The gene of interest (CFTR gene in the case of cystic fibrosis) is under the control of endogenous E1a promoter or the engineered promoter for phosphoglycerate kinase, depending on the ***vector*** used. An intact CFTR coding sequence without mutations or insertions was constructed from the pkk-CFTR1 plasmid contg. the human gene. Expression of CFTR and safety of the recombinant ***adenoviruses*** were tested in 293 cells, monkey bronchiolar cell line 4MBR-5, primary hamster tracheal epithelial cells, human HeLa and HeLa CF PAC cells, and airway epithelial cells from cystic fibrosis patients. The ***second*** - ***generation*** ***vectors*** showed no evidence of inflammation or cytopathic changes upon infection.

L16 ANSWER 11 OF 13 BIOSIS COPYRIGHT 1996 BIOSIS
AN 94:151479 BIOSIS
DN 97164479
TI ***Second*** ***generation*** ***adenovirus*** ***vectors*** for cystic fibrosis gene therapy.
AU Armentano D; Sookdeo C; White G; Giuggio V; Souza D; Couture L; Cardoza L; Vincent K; Wadsworth S; Smith A
CS Genzyme Corp., One Mountain Rd., Framingham, MA 01701, USA
SO Keystone Symposium on Gene Therapy, Copper Mountain, Colorado, USA, January 15-22, 1994. Journal of Cellular Biochemistry Supplement 0 (18 PART A). 1994. 222. ISSN: 0733-1959
DT Conference
LA English

improvement of Ad ***vectors*** .

L27 ANSWER 2 OF 33 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 1
AN 1995:990538 CAPLUS
DN 124:46841
TI Efficient dual transcomplementation of ***adenovirus*** E1 and ***E4*** regions from a 293-derived cell line expressing a minimal ***E4*** functional unit
AU Yeh, Patrice; Dedieu, Jean-Francois; Orsini, Cecile; Vigne, Emmanuelle; Denefle, Patrice; Perricaudet, Michel
CS Lab. Virus Oncogenes, Cent. Natl. Recherche Scientifique URA, Villejuif, 94805, Fr.
SO J. Virol. (1996), 70(1), 559-65
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB Transgene expression after the administration of recombinant ***adenovirus*** with E1 deleted is constantly transient. It is admitted that E1A-substituting activities of cellular or viral origin allow viral antigen synthesis and trigger cytotoxic lymphocyte-mediated clearance of the recipient cells. Our approach to solving this problem relies on the addnl. deletion of the ***E4*** region from the ***vector*** backbone as this region upregulates viral gene expression at both transcriptional and posttranscriptional levels. As a prerequisite to the construction of E1 ***E4*** doubly defective ***adenoviruses*** , we investigated the possibility of transcomplementing both functions within a single cell. In particular, the distal ORF6 + ORF7 segment from the ***E4*** locus of ***adenovirus*** type 5 was cloned under the control of the dexamethasone-inducible mouse mammary tumor virus long terminal repeat. Following transfection into 293 cells, clone IGRP2 was retained and characterized as it can rescue the growth defect of all E1+ ***E4*** - ***adenoviral*** deletants tested. DNA and RNA anal. expts. verified that the mouse mammary tumor virus promoter drives the expression of the ORF6 + ORF7 unit and permits its bona fide alternative splicing, generating ORF6/7 mRNA in addn. to the ORF6-expressing primary transcript. Importantly, IGRP2 cells sustain cell confluence for a period longer than that of 293 parental cells and allow the plaque purifn. of E1- or ***E4*** - defective viruses. The dual expression of E1 and ***E4*** regulatory genes within IGRP2 cells is demonstrated by the construction, plaque purifn., and helper-free propagation of recombinant lacZ-encoding doubly defective ***adenoviruses*** harboring different ***E4*** deletions. In addn., the emergence, if any, of replicative particles during viral propagation in this novel packaging cell line will be drastically impaired as only a limited segment of ***E4*** has been integrated.

L27 ANSWER 3 OF 33 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 2
AN 1995:990534 CAPLUS
DN 124:75678
TI Transduction with recombinant ***adeno*** -associated virus for gene therapy is limited by leading-strand synthesis
AU Fisher, Krishna J.; Gao, Guang-Ping; Weitzman, Matthew D.; DeMatteo, Ronald; Burda, John F.; Wilson, James M.
CS Inst. Human Gene Therapy, Dep. Mol. Cell. Eng., Univ. Pennsylvania Health System, Philadelphia, PA, 19104, USA
SO J. Virol. (1996), 70(1), 520-32
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB ***Adeno*** -assocd. virus is an integrating DNA parvovirus with the potential to be an important vehicle for somatic gene therapy. A potential barrier, however, is the low transduction efficiencies of recombinant ***adeno*** -assocd. virus (rAAV) ***vectors*** . The authors show in this report that ***adenovirus*** dramatically enhances rAAV transduction in vitro in a way that is dependent on expression of early region 1 and 4 (E1 and ***E4*** , resp.) genes and directly proportional to the appearance of double-stranded replicative forms of the rAAV genome. Expression of the open reading frame 6 protein from ***E4*** in the absence of E1 accomplished a similar but attenuated effect. The helper activity of ***adenovirus*** E1 and ***E4*** for rAAV gene transfer was similarly demonstrated in vivo by using murine models of liver- and lung-directed gene therapy. The data indicate that conversion of a single-stranded rAAV genome to a duplex intermediate limits transduction and usefulness for gene therapy.

L27 ANSWER 5 OF 33 CAPLUS COPYRIGHT 1996 ACS
AN 1995:996825 CAPLUS
DN 124:47633
TI ***Adenovirus*** supervector system for heterologous DNA transfer, gene tissue-specific expression in mammal, and gene therapy
IN Zhang, Wei-Wei; Roth, Jack
PA Board of Regents, University of Texas System, USA
SO PCT Int. Appl., 73 pp.
CODEN: PIXXD2
PI WO 9527071 A2 951012
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-US4138 950404
PRAI US 94-222285 940404
DT Patent
LA English
AB An ***adenoviral*** supervector system is disclosed that is capable of expressing more than 7.5 kilobases of heterologous DNA in a replication defective ***adenoviral*** ***vector*** . The supervector system comprises an ***adenoviral*** ***vector*** construct and a helper cell. The ***vector*** construct is capable of being replicated and packaged into a virion particle in the helper cell. In particular, the helper cell expresses DNA from the E2 region of the ***adenovirus*** 5 genome and complements deletions in that region in the ***vector*** construct. In certain embodiments, the disclosed invention comprises tissue specific expression of up to 30 kb of heterologous DNA directed by an ***adenoviral*** ***vector*** . Also disclosed are methods of transferring heterologous DNA into mammalian cells.

L27 ANSWER 6 OF 33 CAPLUS COPYRIGHT 1996 ACS
AN 1995:951301 CAPLUS
DN 123:332111
TI Integrative ***adenovirus*** expression ***vectors*** for use in gene therapy
IN Denefle, Patrice; Latta, Martine; Perricaudet, Michel; Vigne, Emmanuelle
PA Rhone-Poulenc Rorer S.A., Fr.
SO PCT Int. Appl., 49 pp.
CODEN: PIXXD2
PI WO 9523867 A1 950908
DS W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-FR233 950228
PRAI FR 94-2445 940303
DT Patent
LA French
AB Recombination-defective ***adenoviruses*** carrying a cassette that can be integrated into the genome of host cells are constructed for use in gene therapy. The cassette particularly contains at least one inverted terminal repeat (ITR) of an ***adeno*** -assocd. virus (AAV) and a therapeutic gene. The use of the AAV ITR directs integration to the same locus in all cases and minimizes possible complications from random integration. The construction of virus carrying the lacZ reporter gene or a human lipoprotein AI gene under control of viral (vesicular stomatitis or Rous sarcoma virus) promoters is described.

L27 ANSWER 7 OF 33 CAPLUS COPYRIGHT 1996 ACS
AN 1995:468701 CAPLUS
DN 122:207001
TI Replication-defective ***adenovirus*** ***vectors*** capable of carrying very large DNA inserts for use in gene therapy
IN Perricaudet, Michel; Vigne, Emmanuelle; Yeh, Patrice
PA Rhone-Poulenc Rorer S.A., Fr.
SO PCT Int. Appl., 44 pp.
CODEN: PIXXD2
PI WO 9502697 A1 950126
DS W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 94-FR851 940708

PRAI FR 93-8596 930713
FR 94-4590 940418
DT Patent
LA French
AB Novel ***adenovirus*** -derived viral ***vectors*** that carry very little of the ***adenovirus*** genome and that can carry large DNA inserts are described for use in gene therapy. The ***vectors*** retain functional inverted terminal repeats and encapsidation signals but lack a functional E1 gene with the genes E2, ***E4*** , and L1-L5 optionally also inactivated. Packaging cell lines are also described. Expression constructs may use inducible viral promoters such as the glucocorticoid-inducible promoter of the mouse mammary tumor virus long terminal repeat.

L27 ANSWER 8 OF 33 CAPLUS COPYRIGHT 1996 ACS
AN 1995:435870 CAPLUS
DN 122:180302
TI ***Adenovirus*** -based expression ***vectors*** capable of carrying large DNA inserts for use in gene therapy
IN Michel, Perricaudet; Emmanuelle, Vigne
PA Centre nal Recherc Scientifique, Fr.; Roussy Institut Gustave
SO Fr. Demande, 27 pp.
CODEN: FRXXBL
PI FR 2707664 A1 950120
AI FR 93-8596 930713
DT Patent
LA French
AB ***Adenovirus*** ***vectors*** capable of carrying up to 30 kb of foreign DNA and of forming stable transformants are described for use in gene therapy. These ***vectors*** carry very little of the ***adenovirus*** genome and so are poorly immunogenic, pathogenic, and transmissible and do not replicate or recombine strongly. The virus retains ITR and encapsidation sequences and the E1 gene and at least one of E2, ***E4*** , and L1-L5 are are non-functional. Animal cell lines carrying genes complementing the mutation are used to package the virus. The genes are under control of an inducible non- ***adenovirus*** promoter such as the glucocorticoid-inducible LTR promoter of mouse mammary tumor virus.

L27 ANSWER 10 OF 33 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 5
AN 1996:93096 CAPLUS
TI Development of cell lines capable of complementing E1, ***E4*** , and protein IX defective ***adenovirus*** type 5 mutants
AU Krougliak, Valeri; Graham, Frank L.
CS Department Biology, McMaster University, Hamilton, Ont., ON, L8S 4K1, Can.
SO Hum. Gene Ther. (1995), 6(12), 1575-86
CODEN: HGTHE3; ISSN: 1043-0342
DT Journal
LA English
AB The cloning capacity of currently available E1- and E3-deleted ***adenovirus*** (Ad) ***vectors*** does not exceed 8 kb. To increase capacity and improve ***vector*** safety further, we have explored the possibility that Early Region 4 (***E4***) and the gene encoding protein IX (pIX) might also be deleted. To generate cell lines expressing sufficient levels of ***E4*** and pIX proteins in trans in addn. to E1-encoded proteins to complement mutations in these genes, we transformed 293 cells with constructs contg. the ***E4*** transcription unit and pIX coding sequences under the control of inducible mouse mammary tumor virus (MMTV) and metallothionein promoters, resp. We obtained two lines, VK2-20 and VK10-9, that express both ***E4*** and pIX proteins as well as E1. The lines could be efficiently transfected with DNA, and allowed the rescue and propagation of an ***adenovirus*** recombinant, Ad5dlE3,4, contg. a 2.7-kb E3 deletion and a 2.8-kb ***E4*** deletion in addn. to an insertion of plasmid DNA sequences in E1A. Because the ***E4*** sequences within VK2-20 and VK10-9 cells do not overlap with the DNA sequence of Ad5dlE3, ***E4*** , the probability of regeneration of the wild-type ***E4*** during virus propagation should be very low. Using the cell lines described in this study, it should be possible to generate Ad ***vectors*** lacking E1, pIX, E3, and ***E4*** . This would not only increase capacity over that of currently available ***vectors*** (to .apprx.11 kb) but would also result in more severely attenuated ***vectors*** than those with deletions only of E1 or of E1 and E3 and, hence, safer for use in gene therapy protocols.

L27 ANSWER 11 OF 33 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 6
AN 1995:979457 CAPLUS
DN 124:77733
TI Characterization of an ***adenovirus*** gene transfer ***vector*** containing an ***E4*** deletion
AU Armentano, Donna; Sookdeo, Cathleen C.; Hehir, Kathleen M.; Gregory, Richard J.; George, Judith A. St.; Prince, Gregory A.; Wadsworth,

Samuel C.; Smith, Alan E.
CS Genzyme Corporation, Framingham, MA, 01701, USA
SO Hum. Gene Ther. (1995), 6(10), 1343-53
CODEN: HGTHE3; ISSN: 1043-0342
DT Journal
LA English
AB We describe the construction and characterization of an ***adenovirus*** type 2 ***vector*** , Ad2E4ORF6, which has been modified in the ***E4*** region to contain only open reading frame 6. When assayed in cultured cells, Ad2E4ORF6 virus replication is slightly delayed but viral DNA synthesis, host-cell protein synthesis shut-off, and virus yield are indistinguishable from wild type. Late protein synthesis is normal with the exception of fiber synthesis, which is reduced approx. 10-fold. Despite the reduced fiber synthesis, Ad2E4ORF6 viral particles appear to contain a full complement of fiber protein. Virus replication in cotton rats indicates that Ad2E4ORF6 is replication defective in vivo. This may have safety implications for the development of ***adenovirus*** ***vectors*** in that virus arising by recombination in the E1 region of an Ad2E4ORF6-based ***vector*** would be defective for growth in vivo. The deletion of ***E4*** open reading frames that are not required for virus growth in vitro increases the cloning capacity of ***adenovirus*** ***vectors*** by 1.9 kb and may be generally useful for the construction of ***adenovirus*** ***vectors*** contg. large cDNA inserts and/or regulatory elements. We describe the inclusion of the A2E4ORF6 modification in a recombinant ***adenovirus*** ***vector*** , Ad2/CFTR-2, for gene transfer of the human cystic fibrosis transmembrane regulator (CFTR).

L27 ANSWER 12 OF 33 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 7
AN 1995:1007443 CAPLUS
DN 124:47605
TI A packaging cell line for propagation of recombinant ***adenovirus*** ***vectors*** containing two lethal gene-region deletions
AU Wang, Q.; Jia, X.-C.; Finer, M. H.
CS Cell Genesys Inc., Foster City, CA, 94404, USA
SO Gene Ther. (1995), 2(10), 775-83
CODEN: GETHEC; ISSN: 0969-7128
DT Journal
LA English
AB A cell line that provides the E1 as well as the ***E4*** gene functions of human ***adenovirus*** 5 has been established by introduction of the full-length Ad5 ***E4*** region into 293 cells. To avoid the E1A transactivation of the ***E4*** gene expression, the ***E4*** promoter was replaced by the mouse

.alpha. inhibin promoter contg. a cAMP response element. This cell line was used to generate E1/ ***E4*** -deleted ***adenovirus*** ***vectors*** contg. a lacZ gene in the E1 region under the control of mouse pgk promoter. The titer and the lazZ gene expression of E1/ ***E4*** -deleted ***adenovirus*** ***vector*** were comparable to those of E1-deleted ***vectors*** . Evidence of cytopathic effect was absent following infection of nonpermissive cell lines with E1/ ***E4*** -deleted ***adenovirus*** in vitro. Establishment of the 293- ***E4*** cell line and the generation of E1/E1-deleted ***adenovirus*** ***vectors*** may prolong gene expression in vivo and significantly improve the safety of ***adenovirus*** ***vectors*** for human gene therapy.

L27 ANSWER 18 OF 33 CAPLUS COPYRIGHT 1996 ACS
AN 1995:446669 CAPLUS
DN 122:232658
TI Replication-defective ***adenoviruses*** for use in gene therapy and complementing cell lines for use in propagation and packaging of the virus
IN Imler, Jean-Luc; Methali, Majid; Pavirani, Andrea
PA Transgene S.A., Fr.
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
PI WO 9428152 A1 941208
DS W: AU, CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-FR624 940527
PRAI FR 93-6482 930528
DT Patent
LA French
AB Novel defective ***adenoviruses*** for the transfer and expression of an exogenous nucleotide sequence in a host cell or organism are described. Novel cell lines complementing the defective ***adenovirus*** for use in the prepn. of these novel defective ***adenoviruses*** and their use in therapeutics are also described. These viruses have deletions in the E1A region in combination with deletions in the E1B or E2 and ***E4*** regions in combination, the E3 region or the encapsidation site. The construction of deletion mutants of Ad5 and of cell lines carrying the genes deleted from the corresponding virus for propagation and packaging are described. The E1A gene in these lines is placed under the control of an externally-regulated promoter such as yeast GAL4.

L27 ANSWER 19 OF 33 CAPLUS COPYRIGHT 1996 ACS
AN 1994:595933 CAPLUS
DN 121:195933
TI Gene therapy for cystic fibrosis with ***adenovirus*** -based ***vectors*** encoding the CFTR protein
IN Gregory, Richard J.; Armentano, Donna; Couture, Larry A.; Smith, Alan E.
PA Genzyme Corp., USA
SO PCT Int. Appl., 168 pp.
CODEN: PIXXD2
PI WO 9412649 A2 940609
DS W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US11667 931202
PRAI US 92-985478 921203
US 93-130682 931001
US 93-136742 931013
DT Patent
LA English
AB ***Adenovirus*** -based ***vectors*** are disclosed for use in gene therapy, esp. for cystic fibrosis. Advantages of ***adenovirus*** -based ***vectors*** for gene therapy are (1) they appear to be relatively safe, (2) can be manipulated to encode the desired gene product, (3) at the same time are inactivated in terms of their ability to replicate in a normal lytic viral life cycle, and (4) have a natural tropism for airway epithelia. One such ***adenovirus*** -based ***vector*** comprises an ***adenovirus*** 2 serotype genome in which the E1a and E1b regions were deleted and replaced by genetic material of interest (e.g., DNA encoding the cystic fibrosis transmembrane regulator protein). The ***vectors*** can also encompass pseudo-***adenoviruses*** (PAV), which comprise ***adenovirus*** 2 inverted repeats and the minimal sequences of a wild-type ***adenovirus*** type 2 genome necessary for efficient replication and packaging. PAVs contain no potentially harmful viral genes, have a theor. capacity of foreign material of nearly 36 kb, may be produced in reasonably high titers and maintain the tropism of the parent ***adenovirus*** for dividing and non-dividing human target cell types. Such a second-generation ***vectors*** contains the open reading frame 6 (ORF6) of ***adenovirus*** early region 4 (***E4***) and is deleted for all other ***E4*** open reading frames. Optionally this ***vector*** can include deletions in the E1 and/or E3 regions. Alternatively, the ***adenovirus*** -based gene therapy ***vector*** contains the ORF3 of ***adenovirus*** ***E4*** and is deleted for all other ***E4*** open reading frames; this

vector can also include deletions in the E1 and/or E3 regions. The deletion of non-essential open reading frames of ***E4*** increases the cloning capacity by .apprx.2 kb without significantly reducing the viability of the virus in cell culture. The gene of interest (CFTR gene in the case of cystic fibrosis) is under the control of endogenous E1a promoter or the engineered promoter for phosphoglycerate kinase, depending on the ***vector*** used. An intact CFTR coding sequence without mutations or insertions was constructed from the pkk-CFTR1 plasmid contg. the human gene. Expression of CFTR and safety of the recombinant ***adenoviruses*** were tested in 293 cells, monkey bronchiolar cell line 4MBR-5, primary hamster tracheal epithelial cells, human HeLa and HeLa CF PAC cells, and airway epithelial cells from cystic fibrosis patients. The second-generation ***vectors*** showed no evidence of inflammation or cytopathic changes upon infection.

L38 ANSWER 17 OF 53 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 12
AN 1994:531289 CAPLUS
DN 121:131289
TI Enhanced expression of p53 in human cells infected with mutant ***adenoviruses***
AU Grand, Roger J. A.; Grant, Michael L.; Gallimore, Phillip H.
CS Med. Sch., Univ. Birmingham, Birmingham, B15 2TT, UK
SO Virology (***1994***), 203(2), 229-40
CODEN: VIRLAX; ISSN: 0042-6822
DT Journal
LA English
AB The expression of p53 in human cells infected with wild-type (wt) and mutant ***adenoviruses*** has been examd. With wt Ad5 and Ad12, and Ad12 viruses carrying lesions in the E1A or the 19 K E1B genes, there was a pronounced decrease in the level of p53 during the course of infection. However, when cells were infected with mutant viruses which did not express the larger E1B proteins (Ad12 d/620 and Ad5 d/338 and pm381) the concn. of p53 increased markedly to levels comparable to those seen in ***adenovirus*** transformed cells. This increase in level of p53 correlated closely with the advent of E1A expression. Infection with Ad5 d/355 (which carries a lesion in the ***E4*** gene) also resulted in an increase in p53 expression. The authors have concluded that these results can be explained on the basis of the known ability of E1A to stabilize p53 and of the E1B 58 K: ***E4*** 34 K protein complex to regulate mRNA metab. during viral infection, although large increases in expression of p53 or any other cellular proteins following infection with these viruses have not previously been reported. It is suggested that the high concns. of p53 could explain the inability of 54 K and 58 K neg. mutants to transform cells in culture. In cells infected with d/355 both the Ad5 E1B 58 K protein and p53 were located in the nucleus. It was shown by coimmunopptn. expts. that these proteins formed a complex which was stable in the presence of high concns. of NaCl. The interaction of the Ad12 E1B 54 K protein and p53 has also been demonstrated in Ad12 E1-transformed cells by immunopptn. expts. These data, taken in conjunction with previous results, have suggested that increased expression of p53 is unrelated to complex formation with the larger Ad E1B proteins.

L38 ANSWER 18 OF 53 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 13
AN 1994:98123 CAPLUS
DN 120:98123
TI Deletion of the ***E4*** region of the genome produces ***adenovirus*** DNA concatemers
AU Weiden, Michael D.; Ginsberg, Harold S.

CS Coll. Phys. Surg., Columbia Univ., New York, NY, 10032, USA
SO Proc. Natl. Acad. Sci. U. S. A. (***1994***), 91(1), 153-7
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Two mutants contg. large deletions in the ***E4*** region of the ***adenovirus*** genome H5dL808 (93.0-97.1 map units) were used to investigate the role of ***E4*** genes in ***adenovirus*** DNA synthesis. Infection of KB human epidermoid carcinoma cells with either mutant resulted in prodn. of large concatemers of viral DNA. Only monomer viral genome forms were produced, however, when mutants infected W162 cells, a monkey kidney cell line transformed with and expressing the ***E4*** genes. Diffusible ***E4*** gene products, therefore, complement the ***E4*** mutant phenotype. The viral DNA concatemers produced in dL366- and dL808-infected KB cells did not have any specific orientation of monomer joining: the junctions consisted of head-to-head, head-to-tail, and tail-to-tail joints. The junctions were covalently linked mols., but mols. were not precisely joined, and restriction enzyme maps revealed a heterogeneous size distribution of junction fragments. A series of mutants that disrupted single ***E4*** open reading frames (ORFs) was also studied: none showed phenotypes similar to that of dL366 or dL808. Mutants contg. defects in both ORF3 and ORF6, however, manifested the concatemer phenotype, indicating redundancy in genes preventing concatemer formation. These data suggest that the ***E4*** ORFs 3 and 6 express functions crit. for regulation of viral DNA replication and that concatemer intermediates may exist during ***adenovirus*** DNA synthesis.

L38 ANSWER 35 OF 53 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 23
AN 1993:227403 CAPLUS
DN 118:227403
TI ***Adenovirus*** early region 4 and viral DNA synthesis
AU Bridge, Eileen; Medghalchi, Susan; Ubol, Sukithida; Leesong, Minsun; Ketner, Gary
CS Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205, USA
SO Virology (***1993***), 193(2), 794-801
CODEN: VIRLAX; ISSN: 0042-6822
DT Journal
LA English
AB Mutants of human ***adenovirus*** type 5 (Ad5) lacking early region 4 (***E4***) display a complex phenotype that includes a delay in the onset of viral DNA replication in low-multiplicity infections. Studies of viral DNA replication in vitro have not revealed a requirement for ***E4*** products in DNA synthesis and, for most ***E4*** mutants, defects in DNA replication are not apparent at high multiplicities of infection. The effects of ***E4*** mutations on DNA replication therefore may reflect a role for ***E4*** in the regulation of replication rather than in the process of DNA synthesis. The ***E4*** mutant H5dl1014 carries two deletion mutations that together destroy all ***E4*** open reading frames (ORFs) except ORF 4. Immunopptn. measurements of the level of the ORF 4 product confirm that H5dl1014 accumulates the ORF 4 product in somewhat larger amts. than wild-type Ad5. H5dl1014 is profoundly defective in viral DNA replication at a multiplicity of infection (50 PFU/cell) and time (24 h after infection) that permit mutants lacking all seven ***E4*** products to accumulate approx. normal amts. of DNA. In contrast, H5dl1019, a deriv. of H5dl1014 in which the expression of ORF 4 is prevented by a mutation in the ORF 4 ATG initiator codon, produces DNA normally under these conditions. The product of ORF 4 therefore is necessary for the inhibition of viral DNA replication in H5dl1014-infected cells. H5dl1014 also inhibits, in trans, the synthesis of viral DNA by other ***E4*** mutants that lack both ***E4*** ORFs 3 and 6. Viruses that possess either of those ORFs are not subject to inhibition, indicating that the ORF 3 and 6 products antagonize the effect of ORF 4. These observations are consistent with a regulatory role for the ***E4*** ORF 3, 4, and 6 products in viral DNA replication in ***adenovirus*** -infected cells.

L49 ANSWER 4 OF 19 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 4
AN 1995:1007443 CAPLUS
DN 124:47605
TI A packaging cell line for propagation of recombinant adenovirus vectors containing two lethal gene-region deletions
AU Wang, Q.; Jia, X.-C.; Finer, M. H.
CS Cell Genesys Inc., Foster City, CA, 94404, USA
SO Gene Ther. (1995), 2(10), 775-83
CODEN: GETHEC; ISSN: 0969-7128
DT Journal
LA English
AB A cell line that provides the E1 as well as the E4 gene functions of human adenovirus 5 has been established by introduction of the full-length Ad5 E4 region into 293 cells. To avoid the E1A transactivation of the E4 gene expression, the E4 ***promoter*** was replaced by the mouse . ***alpha*** . ***inhibin*** ***promoter*** contg. a cAMP response element. This cell line was used to generate E1/E4-deleted adenovirus vectors contg. a lacZ gene in the E1 region under the control of mouse pgk ***promoter*** . The titer and the lazZ gene expression of E1/E4-deleted adenovirus vector were comparable to those of E1-deleted vectors. Evidence of cytopathic effect was absent following infection of nonpermissive cell lines with E1/E4-deleted adenovirus in vitro. Establishment of the 293-E4 cell line and the generation of E1/E1-deleted adenovirus vectors may prolong gene expression in vivo and significantly improve the safety of adenovirus vectors for human gene therapy.

L49 ANSWER 8 OF 19 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 7
AN 1994:236398 CAPLUS
DN 120:236398
TI Structure of the . ***alpha*** .- ***inhibin*** gene and its regulation in the ruminant gonad: inverse relationship to oxytocin gene expression
AU Ungefroren, Hendrik; Wathes, D. Claire; Walther, Norbert; Ivell, Richard
CS Inst. Horm. Fertil. Res., Univ. Hamburg, Germany
SO Biol. Reprod. (1994), 50(2), 401-12
CODEN: BIREBV; ISSN: 0006-3363
DT Journal
LA English
AB The genes for the . ***alpha*** . subunit of ***inhibin*** and for the nonapeptide hormone oxytocin are both expressed in the granulosa cells of the ruminant follicle as well as in the Sertoli cells of the ruminant testis. Northern hybridization of mRNA from both ovary and testis indicate that in both gonads the expression of the 2 genes is inversely regulated. In the luteinizing granulosa cells, in vitro as in vivo, the . ***alpha*** .- ***inhibin*** gene is down-regulated when the oxytocin gene is up-regulated. In the Sertoli cells of the bull and sheep testis, the situation is similar, with the . ***alpha*** .-inhibiin gene being up-regulated in the prepubertal gonad and down-regulated concomitantly with an up-regulation of the oxytocin gene in early puberty. The gene for the bovine . ***alpha*** .- ***inhibin*** subunit was cloned and characterized. Assessment of transcriptional initiation by primer extension and RNase protection assays showed that several different sites were used in both granulosa cells and testis. Transient transfection of primary bovine granulosa cells with . ***alpha*** .- ***inhibin*** /luciferase gene constructs indicated that a major ***promoter*** element resided in the region -178 to -245 resp. to the methionine start codon of translation, a region that contains a cAMP response element. The ability of forskolin to up-regulate the transcription of transfected gene constructs also depended on the integrity of this region. In contrast, transfection of TM4 cells led to transcriptional initiation from a unusual site in the . ***alpha*** .- ***inhibin*** gene and to a lack of forskolin regulation. Comparison of the . ***alpha*** .- ***inhibin*** and oxytocin genes indicates that although both can be up-regulated by FSH or by forskolin within the same cells, different mechanisms of signal transduction are involved to explain the temporal differences in expression. Together the results indicate that a differentiation step occurring in Sertoli cells at early puberty and in granulosa cells at luteinization involves comparable regulation of genes

through the sequential action of different cAMP-linked transcription factors.

L49 ANSWER 9 OF 19 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 8
AN 1994:550494 CAPLUS
DN 121:150494
TI Negative control of the rat ***inhibin*** . ***alpha*** . subunit ***promoter*** in MA-10 Leydig tumor cells
AU Feng, Z.-M.; Chen, C.-L. C.
CS Population Council, New York, NY, 10021, USA
SO J. Mol. Endocrinol. (1994), 13(1), 39-47
CODEN: JMLEEI; ISSN: 0952-5041
DT Journal
LA English
AB The ***promoter*** /regulatory sequences responsible for the transcription of the rat ***inhibin*** . ***alpha*** . subunit gene in the testis were identified by the transient expression in an MA-10 Leydig tumor cell line of a bacterial reporter gene, chloramphenicol acetyltransferase (CAT), which was driven by different regions of the 5'-flanking sequence of the ***inhibin*** . ***alpha*** . subunit gene. The CAT activity was elevated when the 2.0-kb 5'-flanking . ***alpha*** . subunit gene fragment was progressively shortened from its 5' end, and a maximal increase was reached when the CAT gene was driven by an . ***alpha*** . subunit gene ***promoter*** extending to -163 bp. This construct was termed A. ***alpha*** .BstCAT. Furthermore, when either the -2.0 to -1.6 kb or the -2.0 to -1.0 kb . ***alpha*** . subunit DNA fragment was fused to A. ***alpha*** .BstCAT, the CAT activity was markedly suppressed, indicating the presence of neg. regulatory DNA elements (NREs) in the upstream region of the gene. The cAMP responsiveness of the . ***alpha*** . subunit gene, which was dependent upon the putative cAMP response element within the 67 bp . ***alpha*** . subunit ***promoter*** , was not affected by the upstream NREs. The inhibitory effect was also demonstrated when the -2.0 to -1.0 kb fragment was placed in either orientation with respect to the . ***alpha*** . subunit ***promoter*** or to a thymidine kinase ***promoter*** , suggesting that the NRE(s) can act as a silencer. Based on the authors' observations the authors conclude that the basal expression of the rat ***inhibin*** . ***alpha*** . subunit gene in testicular MA-10 cells may, at least in part, be controlled by the upstream silencer(s) and NRE(s).

L49 ANSWER 10 OF 19 MEDLINE

L49 ANSWER 11 OF 19 CAPLUS COPYRIGHT 1996 ACS
AN 1994:125198 CAPLUS
DN 120:125198
TI Isolation and characterization of mouse ***inhibin*** . ***alpha*** . gene and its ***promoter*** : stimulation by activin and follicle-stimulating hormone
AU Su, Jyan Gwo Joseph
CS Univ. California, San Diego, CA, USA
SO (1992) 229 pp. Avail.: Univ. Microfilms Int., Order No. DA9306367 From: Diss. Abstr. Int. B 1993, 53(11), 5564
DT Dissertation
LA English
AB Unavailable

L49 ANSWER 13 OF 19 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 9
AN 1993:248772 CAPLUS
DN 118:248772
TI Characterization of mouse ***inhibin*** . ***alpha*** . gene and its ***promoter***
AU Su, Jyan Gwo J.; Hsueh, Aaron J. W.
CS Med. Cent., Stanford Univ., Stanford, CA, 94305-5317, USA
SO Biochem. Biophys. Res. Commun. (1992), 186(1), 293-300
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
AB ***Inhibin*** suppresses the pituitary secretion of FSH but not LH. The 2 forms of ***inhibin*** are composed of a common . ***alpha*** . subunit linked to either a .beta.A or .beta.B subunit. The mouse ***inhibin*** . ***alpha*** . gene was isolated and shown to have 2 exons spanning a 1.7 Kb intron. The proximal 5' flanking region has neither TATA and CAAT boxes nor GC-rich area. Using the 5' flanking region of mouse ***inhibin*** . ***alpha*** . gene linked to the luciferase gene, transfection of rat granulosa cells indicated that the first 165 bp of the ***promoter*** region is required for basal expression. The mouse ***inhibin*** . ***alpha*** . genomic clone should be useful for anal. of hormonal control of ***inhibin*** . ***alpha*** . transcription and the generation of mice with targeted deletion of this gene.

L49 ANSWER 16 OF 19 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 11
AN 1990:133640 CAPLUS
DN 112:133640
TI Cloning and characterization of the rat . ***alpha*** .- ***inhibin*** gene
AU Albiston, Anthony L.; Lock, Peter; Burger, Henry G.; Krozowski, Zygmunt S.
CS Med. Res. Cent., Prince Henry's Hosp., Melbourne, 3004, Australia
SO Mol. Cell. Endocrinol. (1990), 68(2-3), 121-8
CODEN: MCEND6; ISSN: 0303-7207
DT Journal
LA English
AB The gene for the rat glycoprotein hormone . ***alpha*** .- ***inhibin*** was cloned and characterized. The entire gene was contained within a 5.5-kilobase EcoRI fragment. It is composed of 2 exons sepd. by a 1.5-kb intron. Primer extension and S1 nuclease anal. showed that the major transcription initiation site in the ovary was 77 bp from the start of translation. The ***promoter*** region of the gene did not contain a conventional TATA box, but instead a no. of GA-rich repeated sequences were present. Other potential regulatory elements found included a repeating purine-pyrimidine tract (TG)28, cAMP and phorbol ester-response elements, and a putative glucocorticoid-response element. Southern blot anal. of rat genomic DNA indicated that there is a single gene for . ***alpha*** .- ***inhibin*** in the rat.

L49 ANSWER 17 OF 19 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 12
AN 1990:472126 CAPLUS
DN 113:72126
TI Analysis of the 5'-flanking regions of rat ***inhibin*** . ***alpha*** .- and .beta.-B-subunit genes suggests two different regulatory mechanisms
AU Feng, Zong Ming; Li, Yi Ping; Chen, Ching Ling C.
CS Popul. Counc., Rockefeller Univ., New York, NY, 10021, USA
SO Mol. Endocrinol. (1989), 3(12), 1914-25
CODEN: MOENEN; ISSN: 0888-8809
DT Journal
LA English
AB The genes encoding rat ***inhibin*** . ***alpha*** .- and .beta.-B-subunits were isolated and characterized. Both genes contain one intron that interrups the region coding for the precursor portion of the . ***alpha*** .- and .beta.-B-subunits. The transcription start sites of . ***alpha*** .- and .beta.-B-subunits gene were detd. by primer extension and nuclease mapping assay using mRNA from rat ovary and testis. Transcription of the . ***alpha*** .-subunit gene initiates predominantly at 3

adjacent sites with similar intensity. Several potential transcription start sites of .beta.-B subunit gene are spread over 150 nucleotides upstream from translation initiation site. Neither of these 2 genes contains obvious TATA or CCAAT boxes. The . ***alpha*** .-subunit gene contain many GA clusters in the ***promoter*** region, whereas the .beta.-.beta.-subunit gene is highly GC rich. Several GGGCGG repeats and their inverted sequences, which are the potential binding sites for transcription factor Sp1, were obsd. at the 5'-end as well as at the coding region of the .beta.-B-subunit gene. The potential cAMP-responsive element CTGCGTCAG was identified in . ***alpha*** .- but not .beta.-B-subunit gene. This sequence is identical to the cAMP- and phorbol ester-inducible DNA fragment found in the human preproenkephalin gene. The different structure of the ***promoter*** region of rat . ***alpha*** .- and .beta.-B-subunit genes and the presence of a potential cAMP-inducible DNA sequence in the . ***alpha*** .- but not the .beta.-B-subunit gene is consistent with the hypothesis that transcription . ***alpha*** .- and .beta.-B-subunit genes in rat is regulated by different mechanisms.

L67 ANSWER 9 OF 30 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 7
AN 1992:544698 CAPLUS
DN 117:144698
TI Transcriptional regulation by a point mutant of ***adenovirus*** -2 E1a product lacking DNA binding activity
AU Zu, You Li; Takamatsu, Yoshiki; Zhao, Mu Jun; Maekawa, Toshio; Handa, Hiroshi; Ishii, Shunsuke
CS Tsukuba Life Sci. Cent., Inst. Phys. Chem. Res., Tsukuba, 305, Japan
SO J. Biol. Chem. (1992), 267(28), 20181-7
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The ***adenovirus*** E1a protein (E1A) regulates transcription through interaction with transcription factors bound to DNA, like ***cAMP*** ***response*** ***element*** BP1/ATF2, or through dissocg. E2F transcription factor complex. However, it was also reported that E1A can bind to DNA (Chatterjee, P. K., et al, 1988), and it is not clear whether DNA binding to E1A is involved in a part of the process of transcriptional regulation of E1A. In this paper, the small region of E1A that is responsible for DNA binding was identified and a point mutant lacking DNA binding activity was constructed. Anal. of deletion mutants of E1A proteins expressed in bacteria showed that a basic region between amino acids 201 and 216 of E1A is essential for DNA binding. Point mutation of arginines at amino acid nos. 205 and 206 to aspartic acids completely abolished the DNA binding activity of E1A. Using this mutant, the requirement of the E1A DNA binding for E1A-dependent transcriptional regulation was examd. trans-Activation of the ***adenovirus*** E4 ***promoter*** and trans-repression of the human c-erbB-2 ***promoter*** by this point mutant were examd. by contransfection expts. Mutations of the E1A DNA-binding domain affected neither the E1A-induced trans-activation nor trans-repression at all. These results give complete proof that the DNA binding activity of E1A is not required for transcriptional regulation by E1A.

L67 ANSWER 11 OF 30 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 9
AN 1992:149278 CAPLUS
DN 116:149278
TI ***Adenovirus*** E1A represses the cyclic AMP-induced transcription of the gene for phosphoenolpyruvate carboxykinase (GTP) in hepatoma cells
AU Kalvakolanu, Dhananjaya V. R.; Liu, Jinsong; Hanson, Richard W.; Harter, Marian L.; Sen, Ganes C.
CS Dep. Mol. Biol., Cleveland Clin. Res. Inst., Cleveland, OH, 44195-5285, USA
SO J. Biol. Chem. (1992), 267(4), 2530-6
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB ***Adenovirus*** infection of hepatoma cells inhibited transcription of the phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) gene and virtually eliminated transcription of a chimeric gene which contained the PEPCK ***promoter*** linked to the structural gene for chloramphenicol acetyltransferase (CAT). This effect is due to the viral protein E1A, since ***adenovirus*** contg. a deletion of the E1A gene did not repress transcription from the PEPCK ***promoter*** . Both the 243R and 289R products of the E1A gene were effective. The conserved region 1 (CR-1) domain of E1A was required for this effect. Treatment of hepatoma cells with 8-bromo- ***cAMP*** or transfection with plasmids coding for the catalytic subunit of protein kinase A, CAAT/enhancer binding protein .alpha. (C/EBP), or Jun, all potent inducers of PEPCK gene transcription, did not relieve the inhibition caused by E1A. This inhibition does not appear to be mediated by major enhancer ***elements*** and in the PEPCK gene since transcription from the PEPCK ***promoter*** contg. block mutations in binding domains for C/EBP and ***cAMP*** regulatory ***element*** binding protein (CREB) was also inhibited by E1A. Transcription of chimeric genes contg. two copies each of the major ***cAMP*** ***response*** domains (CRE-1 and P-3) linked to a neuronal ***promoter*** and fused to the CAT structural gene was stimulated by the catalytic subunit of protein kinase A, but this effect was totally inhibited by E1A. The strong repressive effect of E1A on PEPCK gene transcription seems to involve an interruption of an obligatory interaction between factors which bind to the ***cAMP*** ***response*** ***element*** in the PEPCK ***promoter*** and the TATA box.

L67 ANSWER 12 OF 30 CAPLUS COPYRIGHT 1996 ACS
AN 1991:650469 CAPLUS
DN 115:250469

TI Complete putative metal finger and leucine zipper structures of CRE-BP1 are required for the E1A-induced trans-activation

AU Zu, You Li; Maekawa, Toshio; Matsuda, Shinji; Ishii, Shunsuke

CS Tsukuba Life Sci. Cent., Inst. Phys. Chem. Res., Tsukuba, 305, Japan

SO J. Biol. Chem. (1991), 266(35), 24134-9
CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB The ***adenovirus*** E1A protein stimulates transcription of various genes. Recent expts. using a fusion protein have shown that E1A can function through a specific CRE (***cAMP*** ***response*** ***element***)-binding protein, CRE-BP1 (also designated ATF02), which stimulates the transcription from a CRE-contg. ***promoter*** by homodimer formation or heterodimer formation with C-Jun. In this paper, the functional domains required for mediation of the E1A-induced trans-activation were analyzed using deletion and point mutants of CRE-BP1. The mutation in the putative metal finger structure or leucine zipper structure completely abolished the ability of CRE-BP1 to mediate the E1A-induced trans-activation. Furthermore, overexpression of CRE-BP1 or c-Jun interfered with the E1A-induced trans-activation. These results suggest that the complete putative metal finger structure in the N-terminal region of CRE-BP1 plays an important role for the E1A-induced trans-activation, and the heterodimer of CRE-BP1 with the unidentified protein participates in the interaction with E1A.

L67 ANSWER 18 OF 30 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 12
AN 1990:527674 CAPLUS
DN 113:127674
TI CREB regulation of cellular cyclic AMP-responsive and ***adenovirus*** early ***promoters***
AU Muchardt, Christian; Li, Ching; Kornuc, Masayo; Gaynor, Richard
CS Sch. Med., UCLA, Los Angeles, CA, 90024-1678, USA
SO J. Virol. (1990), 64(9), 4296-305
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB The ***cAMP*** ***response*** ***element*** -binding protein (CREB) has been demonstrated to be a key mediator of cellular ***promoter*** ***response*** to ***cAMP*** . The binding site for this protein in many cellular ***cAMP*** -inducible ***promoters*** (CRE) contains the palindrome sequence TGACGTCA, which contains two half-sites for CREB binding. A related ***promoter*** ***element*** , with the core sequence TGACG, has significant homol. to an AP1-binding site and contains only one half-site for CREB binding. A group of factors known as activating transcription factors (ATF) has been found to bind to the latter and related sequences found upstream of early ***adenovirus*** ***promoters*** induced by E1A, and these factors are highly homologous to the CREB protein. The authors wished to characterize CREB, c-jun, and c-fos binding to these sites in the somatostatin gene (CRE) and in the ***adenovirus*** early region 3 ***promoter*** (E3/ATF). Oligonucleotides complementary to each of these sites were used in gel retardation assays with in vitro-translated CREB protein. These studies indicated that CREB bound primarily as a dimer to both a single and two half-sites, though there was increased affinity to the double compared with the single half-site. The c-jun and c-fos proteins also bound to both the somatostatin CRE- and E3/ATF-binding sites, but CREB did not bind to AP1 recognition sites nor was it capable of forming heterodimers with either c-jun or c-fos. Truncations of the CREB protein, which eliminated regions of the protein contg. consensus sites for phosphorylation by protein kinase A, protein kinase C, and casein kinase II, bound to both the CRE and ATF sites, indicating that these consensus sites were not essential for DNA binding or dimer formation. Transfection of CREB and protein kinase A expression constructs into F9 cells with ***promoters*** contg. either a single or two half-sites for CREB binding indicated that CREB was capable of similar levels of activation of these constructs. However, the fold activation by CREB was higher for constructs contg. a single half-site compared with those contg. two half-sites. These results demonstrate that multiple mechanisms may

regulate CREB binding, including variations in the sequences in the ***promoter*** -binding site and the presence of related DNA-binding proteins.

L37 ANSWER 4 OF 11 MEDLINE
AN 95092190 MEDLINE
TI Negative control of the rat ***inhibin*** alpha subunit ***promoter*** in MA-10 Leydig tumour cells.
AU Feng Z M; Chen C L
CS Population Council, New York, New York 10021..
NC DK-34449 (NIDDK)
SO JOURNAL OF MOLECULAR ENDOCRINOLOGY, (1994 Aug) 13 (1) 39-47.
Journal code: AEG. ISSN: 0952-5041.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9503
AB The ***promoter*** /regulatory sequences responsible for the transcription of the rat ***inhibin*** alpha subunit gene in the testis were identified by the transient expression in an MA-10 Leydig tumour cell line of a bacterial reporter gene, chloramphenicol acetyltransferase (CAT), which was driven by different regions of the 5' flanking sequence of the ***inhibin*** alpha subunit gene. The CAT activity was elevated when the 2.0 kb 5' flanking alpha subunit gene fragment was progressively shortened from its 5' end, and a maximal increase was reached when the CAT gene was driven by an alpha subunit gene ***promoter*** extending to -163 bp. This construct was termed A alpha BstCAT. Furthermore, when either the -2.0 to -1.6 kb or the -2.0 to -1.0 kb alpha subunit DNA fragment was fused to A alpha BstCAT, and CAT activity was markedly suppressed, indicating the presence of negative regulatory DNA elements (NREs) in the upstream region of the gene. The cyclic AMP (cAMP) responsiveness of the alpha subunit gene, which was dependent upon the putative cAMP response element within the 67 bp alpha subunit ***promoter*** , was not affected by the upstream NREs. The inhibitory effect was also demonstrated when the -2.0 to -1.0 kb fragment was placed in either orientation with respect to the alpha subunit ***promoter*** or to a thymidine kinase ***promoter*** , suggesting that the NRE(s) can act as a silencer. Based on our observations we conclude that the basal expression of the rat ***inhibin*** alpha subunit gene in testicular MA-10 cells may, at least in part, be controlled by the upstream silencer(s) and NRE(s).

L34 ANSWER 1 OF 20 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 1
AN 1995:979457 CAPLUS
DN 124:77733
TI Characterization of an adenovirus gene transfer vector containing an E4 deletion
AU ***Armentano, Donna*** ; Sookdeo, Cathleen C.; Hehir, Kathleen M.; Gregory, Richard J.; George, Judith A. St.; Prince, Gregory A.; Wadsworth, Samuel C.; Smith, Alan E.
CS Genzyme Corporation, Framingham, MA, 01701, USA
SO Hum. Gene Ther. (1995), 6(10), 1343-53
CODEN: HGTHE3; ISSN: 1043-0342
DT Journal
LA English
AB We describe the construction and characterization of an adenovirus type 2 vector, Ad2E4ORF6, which has been modified in the E4 region to contain only open reading frame 6. When assayed in cultured cells, Ad2E4ORF6 virus replication is slightly delayed but viral DNA synthesis, host-cell protein synthesis shut-off, and virus yield are indistinguishable from wild type. Late protein synthesis is normal with the exception of fiber synthesis, which is reduced approx. 10-fold. Despite the reduced fiber synthesis, Ad2E4ORF6 viral particles appear to contain a full complement of fiber protein. Virus replication in cotton rats indicates that Ad2E4ORF6 is replication defective in vivo. This may have safety implications for the development of adenovirus vectors in that virus arising by recombination in the E1 region of an Ad2E4ORF6-based vector would be defective for growth in vivo. The deletion of E4 open reading frames that are not required for virus growth in vitro increases the cloning capacity of adenovirus vectors by 1.9 kb and may be generally useful for the construction of adenovirus vectors contg. large cDNA inserts and/or regulatory elements. We describe the inclusion of the A2E4ORF6 modification in a recombinant adenovirus vector, Ad2/CFTR-2, for gene transfer of the human cystic fibrosis transmembrane regulator (CFTR).

L34 ANSWER 4 OF 20 CAPLUS COPYRIGHT 1996 ACS
AN 1994:595933 CAPLUS
DN 121:195933
TI Gene therapy for cystic fibrosis with adenovirus-based vectors encoding the CFTR protein
IN Gregory, Richard J.; ***Armentano, Donna*** ; Couture, Larry A.; Smith, Alan E.
PA Genzyme Corp., USA
SO PCT Int. Appl., 168 pp.
CODEN: PIXXD2
PI WO 9412649 A2 940609
DS W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 93-US11667 931202
PRAI US 92-985478 921203
US 93-130682 931001
US 93-136742 931013
DT Patent
LA English
AB Adenovirus-based vectors are disclosed for use in gene therapy, esp. for cystic fibrosis. Advantages of adenovirus-based vectors for gene therapy are (1) they appear to be relatively safe, (2) can be manipulated to encode the desired gene product, (3) at the same time are inactivated in terms of their ability to replicate in a normal lytic viral life cycle, and (4) have a natural tropism for airway epithelia. One such adenovirus-based vector comprises an adenovirus 2 serotype genome in which the E1a and E1b regions were deleted and replaced by genetic material of interest (e.g., DNA encoding the cystic fibrosis transmembrane regulator protein). The vectors can also encompass pseudo-adenoviruses (PAV), which comprise adenovirus 2 inverted repeats and the minimal sequences of a wild-type adenovirus type 2 genome necessary for efficient replication and packaging. PAVs contain no potentially harmful viral genes, have a theor. capacity of foreign material of nearly 36 kb, may be produced in reasonably high titers and maintain the tropism of the parent adenovirus for dividing and non-dividing human target cell types. Such a second-generation vectors contains the open reading frame 6 (ORF6) of adenovirus early region 4 (E4) and is deleted for all other E4 open reading frames. Optionally this vector can include deletions in the E1 and/or E3 regions. Alternatively, the adenovirus-based gene therapy vector contains the ORF3 of adenovirus E4 and is deleted for all other E4 open reading frames; this vector can also include deletions in the E1 and/or E3 regions. The deletion of non-essential open reading frames of E4 increases the cloning capacity by .apprx.2 kb without significantly reducing the viability of the virus in cell culture. The gene of interest (CFTR

gene in the case of cystic fibrosis) is under the control of endogenous E1a promoter or the engineered promoter for phosphoglycerate kinase, depending on the vector used. An intact CFTR coding sequence without mutations or insertions was constructed from the pkk-CFTR1 plasmid contg. the human gene. Expression of CFTR and safety of the recombinant adenoviruses were tested in 293 cells, monkey bronchiolar cell line 4MBR-5, primary hamster tracheal epithelial cells, human HeLa and HeLa CF PAC cells, and airway epithelial cells from cystic fibrosis patients. The second-generation vectors showed no evidence of inflammation or cytopathic chan

L40 ANSWER 5 OF 5 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 2
AN 1991:623956 CAPLUS
DN 115:223956
TI Precursors of .alpha.- ***inhibin*** modulate follicle-stimulating hormone receptor binding and biological activity
AU Schneyer, Alan L.; Sluss, Patrick M.; Whitcomb, Randall W.; Martin, Kathryn A.; Sprengel, Rolf; Crowley, William F., Jr.
CS Dep. Med., Massachusetts Gen. Hosp., Boston, MA, 02114, USA
SO Endocrinology (Baltimore) (1991), 129(4), 1987-99
CODEN: ENDOAO; ISSN: 0013-7227
DT Journal
LA English
AB Although several forms of monomeric .alpha.- ***inhibin*** have been isolated from follicular fluid, no biol. function has yet been ascribed to these posttranslationally processed forms of the .alpha.-subunit precursor protein. Moreover, previous studies of a FSH receptor binding competitor (FRBC) isolated and characterized from porcine follicular fluid (pFF) suggested certain biochem. similarities between this protein and .alpha.- ***inhibin*** precursors. The hypothesis was investigated that .alpha.- ***inhibin*** and/or its precursors might represent autocrine and/or paracrine modulators of FSH action in the ovary, accounting for some of this FRBC activity and thereby exerting some degree of regulation over follicular maturation. Three sep. sources of .alpha.- ***inhibin*** proteins were investigated for FRBC activity, including pFF, human FF (hFF), and a ***293*** cell line into which the full-length human .alpha.- ***inhibin*** cDNA had been stably transfected. Conditioned medium from these transfected cells contained several forms of .alpha.- ***inhibin*** precursors as well as mature .alpha.- ***inhibin*** , but no .beta.-subunit or intact ***inhibin*** . .alpha.- ***Inhibin*** proteins from all three sources, purified by a variety of methods, including immunoaffinity chromatog. on an anti-.alpha.- ***inhibin*** column, inhibited FSH binding to both natural tissue FSH receptors as well as recombinant rat FSH receptors expressed in ***293*** cells. Furthermore, dimeric ***inhibin*** and activin, medium from untransfected ***293*** cells, and non-.alpha.- ***inhibin*** -contg. purifn. fractions were inactive in either assay. In addn., purified recombinant .alpha.- ***inhibin*** proteins were partial in vitro FSH antagonists in a bioassay in which cAMP generation from ***293*** cells expressing the recombinant FSH receptor is used as an index of FSH biol. activity. These same fractions of hFF contg. FRBC activity did not bind to LH receptors, thereby demonstrating receptor specificity for this activity. Using sodium dodecyl

sulfate-polyacrylamide gel electrophoresis and Western blotting with .alpha.- ***inhibin*** or FRBC antisera, a 57,000 mol. wt. protein was identified in FRBC-active fractions from all three sources, suggesting that the active moiety was the full-length .alpha.- ***inhibin*** precursor protein or a large mol. wt. fragment, but not mature .alpha.- ***inhibin*** . Lastly, all FRBC activity from all three sources was extd. by an .alpha.- ***inhibin*** immunoaffinity column and was recoverable upon elution. These results demonstrate that proteins derived from the .alpha.- ***inhibin*** precursor modulate FSH binding to its receptor as well as its biol. activity. Since .alpha.- ***inhibin*** precursors have been reported in FF at concns. exceeding 2.5 .mu.g/mL, the potency of the FSH antagonism detd. for .alpha.- ***inhibin*** is consistent with a potential physiol. role for .alpha.- ***inhibin*** as an autocrine or paracrine FSH modulator.

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L6 ANSWER 28 OF 32 CAPLUS COPYRIGHT 1996 ACS DUPLICATE 17
AN 1989:418462 CAPLUS
DN 111:18462
TI Complementation of ***adenovirus*** E4 mutants by transient expression of E4 cDNA and deletion plasmids
AU Ketner, Gary; Bridge, Eileen; Virtanen, Anders; Hemstroem, Cartharina; Pettersson, Ulf
CS Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205, USA
SO Nucleic Acids Res. (1989), 17(8), 3037-48
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB Human ***adenovirus*** mutants that carry a large deletion in early region 4 (E4) are severely defective in the synthesis of viral late proteins. Plasmids that carry intact E4 sequences can complement the late protein synthetic defect of such mutants when introduced into infected cells by transfection, presumably due to the transient expression of E4 products. Cells transfected which cDNA clones capable of expressing E4 open reading frame (***ORF***) ***6*** , or deletion mutant clones expected to express either E4 ***ORF*** ***6*** or E4 ORF 3, also complement the mutants' defects. Thus, these E4 ORFs can individually satisfy the requirement for E4 products in viral late gene expression, and function effectively in the absence of other E4 products. Some E4 deletion mutants also exhibit a defect in the prodn. of viral DNA. All of the clones that stimulate gene expression also enhance one such mutant's ability to accumulate viral DNA. Thus, the ORF 3 and ***ORF*** ***6*** products are also individually sufficient to provide an E4 function necessary for normal viral DNA replication in the absence of other E4 products.